

Research Notes

Impacts of Cotyledon Removal on Survival of Blackbrush (*Coleogyne ramosissima*: Rosaceae) Seedlings

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Introduction

Despite the ecological importance of the regeneration stage of plant life histories (Grime 2001), some of the factors affecting seedling survival, including the interaction between seedlings and their herbivores, are not well-understood (Hanley and May 2006). Herbivory is a major cause of seedling mortality for many woody plant species (Hanley 1998; Moles and Westoby 2004; Fenner and Thompson 2005). The ability of seedlings to cope with herbivory is likely affected by seed size, the presence or absence of cotyledons, and the time elapsed since germination (Bonfil 1998).

Because seedlings of woody plant species are small, they can suffer high mortality due to herbivory (Kitajima and Augspurger 1989; Howe 1990; Osunkoya et al. 1992; Asquith et al. 1997; Kitajima 2003). Carbohydrate reserves are important for seedling recovery after tissue loss, especially in shaded understories where photosynthesis is limited (McPherson and Williams 1998; Canham et al. 1999). Cotyledons can support energy and nutrient demands of a young seedling either as photosynthetic or reserve organs (Kitajima 2003). Cotyledons have nutritional value, and it is common to find young seedlings in the field with cotyledons that have been partially or completely removed by insects and browsing vertebrates (Smythe 1978). Such removal of cotyledons before development of the first true leaf is usually fatal (Kitajima 2003). However, even after development of the first leaf, cotyledon loss may greatly reduce seedling survival (Kitajima 2003).

Using greenhouse experiments, I investigated how the timing of cotyledon damage and variation in its magnitude influencing survival of young blackbrush (*Coleogyne ramosissima* Torr.) seedlings through simulated cotyledon herbivory. Specifically, I examined whether differences in the ability of 4-month-old *Coleogyne* seedlings to survive detachment of cotyledons were related to the timing and extent of cotyledon removal in an environmentally controlled greenhouse.

Methods

Seed Collection and Germination

Coleogyne seeds were collected from late June through early August 2006 in Cold Creek Canyon that lies on the eastern slope of the Spring Mountains (36° 25'N, 115° 28'W; 1,250 to 1,405 m in elevation). The Spring Mountains are located approximately 65 km northwest of the Las Vegas Valley in southern Nevada. A total of 500 potentially viable seeds were collected from nearly monospecific *Coleogyne* shrublands.

Coleogyne seeds were placed at room temperature (22° C) for 4–5 months prior to the initiation of the pot trial experiments. These seeds were stored at 4° C for six weeks in the dark (dry-chilling) to obtain maximum germination (Lei 1997). A relatively long, dry-chilling was required for complete removal of dormancy for seeds especially found at high elevations (Meyer and Pendleton 1990).

During the germination period, 20 seeds were placed between two layers of germination blotters moistened with tap water inside a 10-cm diameter Petri dish. Stacks of Petri dishes were placed in transparent zip-loc bags in a cool chamber to decrease evaporative water loss, and water was added as necessary to maintain saturation of blotters during incubation. *Coleogyne* seeds were incubated at 4° C in the dark except for an occasional, brief exposure to fluorescent light. Germination of most seeds was observed 2–3 weeks into the initial experiment. Seed germination was monitored and recorded for 34 days. Radicle emergence greater than 1 mm was the criterion for seed germination, and over 85% of seeds germinated.

Pot Trial Experiments

Five-week old *Coleogyne* seedlings were planted into 65-mm diameter * 350-mm tall, cone-shaped containers. Pots were randomly arranged in a greenhouse from mid-December 2006 through mid-April 2007. Each pot contained one-third of perlite and two-thirds of natural field soil, thoroughly mixed, without adding fertilizers in order to maintain a low soil fertility level. Perlite was used to enhance aeration and drainage (Lei 2004 and 2006).

Prior to planting, care was taken to allocate seedlings by size to various experimental treatments, so that no single treatment had disproportionately large or small seedlings. Pots were lightly moistened with tap water for 17 weeks (four months) in the greenhouse. Initially, seedlings were watered twice a week for the first three weeks, and thereafter once a week until the end of the pot trial experiments. A small amount of liquid fungicide (Banner Maxx Systematic Fungicide) was applied biweekly. Any deaths observed during the first several days were immediately replaced with new *Coleogyne* seedlings. Survivorship percentages among seven treatment groups were recorded, with 30 individuals in each group for a total of 210 seedlings. During the course of study, light and air temperature regime in the greenhouse resembled winter and spring weather conditions in *Coleogyne* shrublands at mid-elevations of southern Nevada.

Herbivory treatments were applied to *Coleogyne* seedlings. These treatments included timing (control, early, and late) of cotyledon removal. The early removal treatment had both cotyledons excised before appearance of the first set of true leaves, while the later removal treatment had both cotyledons excised shortly after development of the first set of leaves.

In addition, cotyledons were excised with scissors as a gradient of 0% (control), 50% (half), and 100% (all). This herbivory treatment was applied 4 and 21 days after planting into a plastic pot to simulate the extent of cotyledon removal by herbivores before and after the emergence of the first set of true leaves. The 50% removal treatment had one cotyledon excised, while the 100% removal treatment had both cotyledons excised. Scissors were sterilized in 70% isopropyl alcohol between clippings in order to minimize pathogen spread among *Coleogyne* seedlings.

Survivorship of *Coleogyne* seedlings was assessed based on the presence of green leaves and growing shoots. Four months after simulated cotyledon herbivory, seedlings were classified into two categories: alive or dead. Surviving seedlings exhibited net growth, whereas dead seedlings exhibited no growth, with brittle, dark brown leaves.

Table 1. Survivorship of *Coleogyne* seedlings and results of Chi-square (X^2) analysis showing the timing of cotyledon damage and variation in its magnitude using greenhouse experiments ($n = 30$ per treatment group in each variable). Survivorship was based on four months after simulated cotyledon herbivory. Statistical significance is determined at $p \leq 0.05$.

Variable	Survival proportion (%)	X^2	p -value
Control	76.7		
Timing of cotyledon removal		4.32	0.0377
Early (before first set of leaves)	0		
Late (after first set of leaves)	40.0		
Extent of removal (before first set of leaves)		6.41	0.0114
Partial (50%)	16.7		
Total (100%)	0		
Extent of removal (after first set of leaves)		4.02	0.0449
Partial (50%)	26.7		
Total (100%)	16.7		

Statistical Analyses

Proportion of *Coleogyne* seedlings that survived four months after simulated cotyledon herbivory was computed. The experimental treatments included timing of cotyledon removal, as well as extent of cotyledon removal before and after the development of the first set of true leaves. Chi-square analysis, with Yate's correction (Analytical Software 2007), was performed to determine significant difference in mortality, with timing and extent of cotyledon removal as main effects. Statistical significance was determined at $p \leq 0.05$.

Results

Coleogyne cotyledons appeared 12 to 17 days after initial seed germination. Serving as photosynthetic leaves, many cotyledons turned green within 9 days after planting into a pot containing soil mix. Cotyledon length and width (dimensions) were substantially greater than true leaf dimensions. In general, the first set of leaves appeared 13 to 19 days after planting into a pot, and was fully developed 5 to 9 more days after initial appearance. At the same time, additional true leaves began to emerge.

Cotyledon excision had more adverse effects on *Coleogyne* seedling survival than the control group. Because some individuals were alive at the end of this study, survival difference among seven experimental treatments was observed and summarized as proportion surviving after four months (Table 1).

Survivorship of *Coleogyne* seedlings was significantly higher when cotyledons remained intact (control) compared to cotyledons severed before development of the first set of true leaves ($p \leq 0.05$; Table 1). Similarly, survivorship of seedlings was significantly higher when cotyledons remained intact compared to partial or total excision of cotyledons regardless of the presence of the first set of true leaves ($p \leq 0.05$; Table 1).

Discussion

Results of this study support and reinforce the role of cotyledons on early seedling survival of *Coleogyne*. Herbivory can be potentially large during the early seedling life (Hanley and May 2006). Simulated herbivory at the cotyledon stage significantly

decreased the probability of *Coleogyne* seedling survival. The timing and extent of cotyledon loss significantly influenced subsequent seedling survival.

Cotyledons apparently contributed to the energy demand of *Coleogyne* seedlings at a very early stage. Resources transferred from cotyledons to the rest of the seedling during this period are important for survival, even after seedlings had fully expanded leaves apparently available for photosynthetic carbon gain (Kitajima 2003). Without cotyledons, the presence of even a small leaf was critical for survival in this study. All seedlings died after both cotyledons were excised before the emergence of the first set of true leaves.

Survival of *Coleogyne* seedlings was significantly reduced by partial or total cotyledon removal, demonstrating that seeds reserves were stored in cotyledons before and several weeks after germination. Partial or total removal of cotyledons can cause death in young seedlings of woody plant species (Mulligan and Patrick 1985; Armstrong and Westoby 1993). Cotyledons would be important for energy acquisition in seedlings (Milberg and Lamont 1997). Vertebrate and invertebrate herbivores (predators) would prefer to excise the cotyledons as early as possible before their food reserves are completely consumed by the seedlings (Bonfil 1998). Seedlings are unable to survive if cotyledons are defoliated 7 to 15 days after germination (Bonfil 1998), which is in agreement with this study.

In the field, *Coleogyne* seedling survival is influenced by a number of factors in southern Utah and Nevada (Meyer and Pendleton 2005). Most mortality took place early in the first year. Early spring mortality was primarily due to animal (rodent) grazing the new sprouts (Meyer and Pendleton 2005). The seedling-eating rodent communities are comprised of a mixture of heteromyid and non-heteromyid species. If seedlings survive this "rodent sieve", drought-related mortality may occur in late May through early June (Meyer and Pendleton 2005). A majority of seedlings that survived the first summer also survived through the second summer (Meyer and Pendleton 2005).

By reducing shrub seedling survival, herbivory can have adverse effects on the establishment of shrubs in successional systems (Meiners and Handel 2000). Microsites and periods where seedlings are exposed to herbivory could be characterized by high seedling mortality, making successful establishment unlikely (Meiners and Handel 2000). When herbivory is low, establishment rates should be high, resulting in the establishment of a cohort of seedlings (Meiners and Handel 2000). A brief period of low herbivory can result in a window of establishment in successional time (Rankin and Pickett 1989; Peroni 1994).

Acknowledgments

I gratefully acknowledge Steven Lei, David Valenzuela, and Shevaun Valenzuela for collecting *Coleogyne* seeds in the field and for setting up various experimental treatments in a greenhouse at College of Southern Nevada (CSN). Helpful comments provided by Steven Lei greatly improved this manuscript.

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